SHORT COMMUNICATION

The relationship between dietary fat composition and plasma cholesterol esterification in man

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Summary

1. Cholesterol esterification has been studied in the plasma of subjects on diets rich in saturated or polyunsaturated fat.

2. The diet rich in polyunsaturated fat was associated with lower rates of plasma cholesterol esterification in vitro. The data suggest that there was a reduction of plasma lecithin–cholesterol acyltransferase activity as well as decreased ability of the lipoprotein substrates of the enzyme to support esterification.

3. On this diet, there was no change in the proportion of the plasma cholesterol esterified but the plasma cholesterol and triglyceride concentrations were reduced.

Key words: cholesterol, cholesteryl esters, lecithin–cholesterol acyltransferase, lipoproteins, triglycerides.

Introduction

Most of the plasma cholesteryl esters in man are probably synthesized within the plasma (Glomset, 1968) under the influence of lecithin–cholesterol acyltransferase (EC 2.3.1.43), but little is known of the factors that regulate plasma LCAT activity. Relationships to several variables have been reported, such as plasma lipid concentrations and body weight (e.g. Monger & Nestel, 1967; Marcel, Fabien & Davignon, 1971; Akanuma, Kuzuya, Hayashi, Ide & Kuzuya, 1973; Miller & Thompson, 1973). Gjone, Nordøy, Blomhoff & Wiencke (1972) reported reduced plasma cholesterol esterification in vitro when polyunsaturated fat was substituted for saturated fat in the diet. However, it is not clear from their study whether there was any change in LCAT activity since their assay reflects changes in both enzyme activity and in its lipoprotein substrates.

To gain information about those factors that regulate plasma LCAT activity, we have studied fifteen subjects on diets rich in saturated and unsaturated fat, using methods designed to detect changes mediated by alterations in enzyme activity and substrate composition.

Methods

Subjects and diets

Fifteen male subjects (aged 20–63 years) were studied as out-patients. The group comprised normal subjects and patients with endogenous hypertriglyceridaemia and hypercholesterolaemia. None was taking drugs affecting lipid metabolism and causes of secondary hyperlipidaemia had been excluded. Both diets consisted of ordinary foodstuffs, and full details are given by Chait, Onitiri, Nicoll, Rabaya, Davies & Lewis (1974). Each dietary period was 7–9 days, one diet having a ratio of polyunsaturated to saturated fat (P/S ratio) of 0.2. During the other dietary
period, saturated fat was replaced by an energy-eqivalent amount of polyunsaturated fat to give a P/S ratio of 2.4. Body weights were constant to ±1 kg on the diets.

**Analyses and assays**

**Lipid.** Triglycerides and total cholesterol were determined in propan-2-ol extracts of plasma by semi-automated methods (Cramp & Robertson, 1968; Technicon procedures N-78 and N-24a). The method of determining the percentage of plasma cholesterol esterified has been described previously (Thompson & Miller, 1973).

**Assays of LCAT.** The estimation of cholesterol esterification in vitro was based on the method of Glomset & Wright (1964) and has been described by Miller & Thompson (1973). Two modifications were made, in that substrate lipoproteins were preincubated with radioactive cholesterol for 4 h and the total incubation volume was reduced to 1 ml. Thus incubation mixtures contained 0.9 ml of substrate (0.8 ml of heat-inactivated plasma and 0.1 ml of a suspension of [4-14C] cholesterol in albumin) and 0.1 ml of active plasma (LCAT source). The mixture was incubated for 3 h, during which esterification was linear. Three series of incubations were performed. All incubations (including blank non-LCAT-containing incubations) were performed in duplicate and results are expressed as nmol of cholesterol esterified per h per ml of LCAT source.

(a) **Series I assays.** These assays were designed to determine the activity of LCAT in the patients' plasmas on the two diets. Active plasma (0.1 ml) from each experimental subject was assayed against 0.9 ml of 'standard substrate' (a pool of heat-inactivated labelled plasma from several normal subjects).

(b) **Series II assays.** The aim of these assays was to determine if alteration in the composition of the dietary fat had influenced the ability of the subjects' plasma samples to act as LCAT substrates. Substrate (0.9 ml), prepared from the plasma of the experimental subjects, was used in assays containing 0.1 ml of 'standard LCAT' (pooled active plasma from several normal subjects).

(c) **Series III assays.** These assays were performed to see if the alterations in substrate efficiency detected in the series II assays were quantitatively sufficiently great to have made an appreciable contribution to the changes observed in the series I assays. 'Standard LCAT' was assayed against 'standard substrate', the latter having been modified by addition of 0.1 ml of heat-inactivated experimental plasma.

**Results**

**Plasma lipids**

The polyunsaturated fat diet was associated with significantly lower \((P<0.001)\) plasma concentrations of cholesterol (mean 5.37 mmol/l, \(\text{SEM} \ 0.28\)) and triglycerides (1.40 mmol/l, \(\text{SEM} \ 0.21\)) than the saturated fat diet (6.41, \(\text{SEM} \ 0.34\), and 2.34, \(\text{SEM} \ 0.42\), respectively). The percentage of plasma cholesterol esterified was essentially the same on the polyunsaturated (71.1, \(\text{SEM} \ 0.7\)) and saturated fat diets (71.5, \(\text{SEM} \ 0.8\)).

**Assays of LCAT**

Results of the LCAT assays (series I–III) are shown in Fig. 1. Data are available for only thirteen subjects in the series II assays and fourteen subjects in the series III assays. The diet rich in polyunsaturated fat was associated with a significant reduction \((P<0.001)\) in esterification in the series I assays from a mean of 27.1 nmol h\(^{-1}\) ml\(^{-1}\) (\(\text{SEM} \ 1.3\)) to 22.6 (\(\text{SEM} \ 1.5\)). In the series II assays esterification was also significantly reduced \((P<0.001)\) from 48.5 (\(\text{SEM} \ 3.0\)) to 37.2 (\(\text{SEM} \ 2.9\)). In the series III assays, however, esterification was very similar \((P>0.1)\) on the diets rich in saturated (mean 22.7, \(\text{SEM} \ 0.4\)) and unsaturated (mean 22.5, \(\text{SEM} \ 0.4\)) fat. Indeed, not only was esterification very similar for a given subject on each diet in the series III assays but there was very little between-subject variation.

**Discussion**

The results of the series II assays show that when the unsaturation of dietary fat is increased there is a reduction in the ability of plasma to support esterification by a given LCAT source. The series I assays point to a reduction in plasma LCAT activity under the same circumstances, but an element of doubt persists because of the small contribution made by the LCAT source to overall substrate composition in these assays. However, in the series III assays, where a 'standard LCAT' source was used and the substrates were similar in composition to those of the
series I assays, there was no significant ‘between-diet’ difference in esterification. Thus it seems there is a genuine reduction in plasma LCAT activity on high P/S diets, though we cannot say whether enzyme or substrate is rate limiting in a given situation.

This phenomenon may prove to be of considerable practical importance in view of the widespread use of high P/S diets in the treatment of hyperlipidaemias and the suggestions that the role of the high-density lipoprotein–LCAT system is to remove cholesterol from cell membranes (Glomset & Norum, 1973) and arterial wall (Rutenberg & Soloff, 1971).

On theoretical grounds, Schumaker & Adams (1970) postulated a decreased requirement for cholesterol esterification when plasma triglyceride turnover is reduced. The present work seems to fit this concept since we have demonstrated reduced esterification on high P/S diets and Chait et al. (1974), studying a similar group of subjects under identical conditions, produced evidence that such diets are associated with reduced secretion or endogenous triglyceride into plasma. This has been confirmed more directly in rats (Morris, Chait, Cohen & France, 1975). Other work (Miller, 1975) suggests that variables influenced by cholestyramine resin therapy, such as hepatic cholesterol synthesis or plasma concentrations of cholesterol or low-density lipoproteins, do not influence esterification. Thus it seems that inter-relationships with triglyceride metabolism deserve further study in future work on the regulation of plasma LCAT activity.

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References


